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Authors: Dell, Ghislaine, Ward, Andrew, Shokrai, Arman, Madej, Andrej, and Engström, Wilhelm

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[REVIEW]

Regulation of the IGF System by Glucocortidoids

Ghislaine Dell[1], Andrew Ward[1], Arman Shokrai[2], Andrej Madej[3] and Wilhelm Engström[2]*

[1]*Developmental Biology Programme, School of Biology and Bochemistry, University of Bath, BATH, UK,*
[2]*Department of Pathology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, PO Box 7028, S-750 07 Uppsala, Sweden and*
[3]*Department of Physiology, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, PO Box 7045, S-750 07 Uppsala, Sweden*

Introduction

Insulin-like growth factors (IGFs) play a pivotal role in promoting embryonic and fetal growth, and display a wider range of developmental and tissue specific expression than any other growth factors. Glucocorticoids exert a variety of anabolic and catabolic effects and are involved in the organisms response to stress. During development, there appears to be a co-ordinated regulation of glucocorticoid biosynthesis and IGF gene expression in the embryo. In particular, attention has been focussed on the role of the glucocorticoid surge just before birth in regulating IGF levels in the embryo. IGFs and glucocorticoids have been known to have corroborating effects on cell proliferation, with glucocorticoids affecting IGFs at a transcriptional level.

The purpose of this review, then, is to collate the current knowledge of how glucocorticoids can effect regulation of IGFs, both directly and via regulation of the IGF receptors and binding proteins, which themselves regulate IGFs and their effects. We show that this regulation is complex and bi-directional, occurring in different tissue types to up- or downregulate levels of IGF or IGF-binding protein transcription or protein levels, intricately linking the glucocorticoids to the insulin like growth factors in the control of cell growth and proliferation.

The insulin-like growth factors and their receptors

The insulin like growth factors IGF I and IGF II are single chain polypeptides with an approximate molecular weight of 7 kDa (Daughaday and Rotwein, 1989). The mature peptides consist of four distinct domains in IGF I as well as in IGF II-A, B, C and D. The A and B chains show strong homology with preproinsulin. Both IGF I and IGF II are produced as prepropeptides that contain a signal peptide as well as a trailer peptide. In IGF I, there are different signal and trailer peptides that combine to yield different precursor molecules. Hence multistep posttranslational processing is required to obtain identical end-products.

IGFs were discovered on the basis of their ability to stimulate cartilage sulphation and to replace the sulphation activity of growth hormone both in *in vivo* and *in vitro* test systems (Salmon and Daughaday, 1957). The biological significance of this finding was rapidly expanded beyond the study of cartilage sulphation to include stimulation of DNA replication, proteoglycan synthesis, glycosamin synthesis, protein synthesis and accumulation, motility and cell survival (see Jones and Clemmons, 1995 for review). Purification and subsequent amino acid sequence determination revealed the existence of two separate molecules that contain 70 and 67 amino acids, respectively. Due to their high degree of homology with insulin they were denominated IGF I and IGF II (Rinderknecht and Humbel, 1978a, 1978b).

IGFs bind to, and act via three different membrane receptors: the type I and type II IGF receptors and the insulin receptor. The affinities and kinetic properties differ among each of the ligand-receptor interactions. The type I IGF receptor has the highest affinity for IGF I and the type II receptor the highest affinity for IGF II. The insulin receptor binds both IGF I and IGF II with low affinity (Nissey and Kiess, 1991; Steele-Perkins *et al.*, 1988; Werner *et al.*, 1991). The type 1 IGF receptor resembles the insulin receptor. It is arheterodimeric transmembrane protein that consists of two alpha and two beta subunits. Ligand binding induces tyrosine specific autophosphorylation of the receptor as well as of cytoplasmic substrate proteins which is followed by a multifaceted biological response. The type I IGF receptor mediated a vast variety of biological effects exerted by the IGFs (de Meyts *et al.*, 1994; Jones and Clemmons, 1995) and recent mouse genetic experiments show that it is the major mitogenic signalling receptor for both IGF I and IGF II in fetal development (Baker *et al.*, 1993). The functional relationship between IGF II and the insulin receptor was unclear for some time, in particular since tumour hypoglycaemia increased the rate of IGF II gene transcription (Schofield *et al.*, 1991). A recent study shed some light on this issue by demonstrating that the insulin receptor

* Corresponding author: Tel. +46-18-67 11 94;
FAX. +46-18-67 35 32.

can mediate the mitogenic messages of IGF II but not of IGF I (Morrione *et al.*, 1997). Furthermore, IGF II mitogenic signalling via the insulin receptor is known to be necessary for full growth of the placenta. In contrast, the type II IGF receptor is a monomeric protein which consists of a major extracellular portion with fifteen repeats of a cysteine rich sequence, as well as a single hydrophobic transmembrane helix and a minor cytoplasmic part. The type II receptor in mammals is also the mannose-6-phosphate receptor (Morgan *et al.*, 1987) and binds IGF II and Mannose-6-phosphate at distinct binding sites on the receptor protein (Braulke *et al.*, 1988). Binding of either of the ligands does not induce a phosphorylation response from this receptor, rather its role appears to be to participate in endocytosis as well as sorting of lysosomal enzymes. The type II receptor is also involved in membrane trafficking through rapid cycling between cytosolic membrane compartments and the plasma membrane. It also induces a redistribution of receptors (Braulke and Mieskes, 1992) as well as modulating insulin exocytosis under physiological conditions (Zhang *et al.*, 1997). In keeping with this cell biology, mouse genetic experiments indicate that the type II receptor acts primarily as a scavenger for IGF II. Loss or inactivation of the type II receptor gene results in a general overgrowth that is ameliorated in the absence of the IGF II ligand (Filson *et al.*, 1993; Wang *et al.*, 1994).

The insulin like growth factors IGF I and IGF II display a wider range of developmental and tissue specific expression than any other known growth factors (Schofield, 1992). It is generally implied that they play a pivotal role in promoting embryonic and fetal growth. Although the IGFs were originally believed to act as a classical hormone, mediating the action of growth hormone, they are now known to act in a paracrine or autocrine fashion. In the fetus and adult, both IGFs are mainly synthesised the liver. However nearly all embryonic tissues express the IGF II gene (Hydahl *et al.*, 1986; Scott *et al.*, 1985). During development many fetal tissues express one or both IGFs with expression detectable from early post implantation and onwards. It is noteworthy that type I IGF receptors are expressed either by the IGF expressing cells or by adjacent cells, which forms a prerequisite for paracrine or autocrine loops.

A large variety of normal and neoplastic cells cultured *in vitro* express the IGF II gene. The level of expression can be influenced by a variety of culture conditions including the serum concentration. In addition to the bona fide 67 amino acid IGF II protein, there are examples of high molecular weight variants produced by cells cultured *in vitro* (Gowan *et al.*, 1987; Granerus *et al.*, 1993; Schofield *et al.*, 1990) that show a different affinity to the IGF receptors (Schofield *et al.*, 1994). The biological implications of these variant IGF II molecules are unclear, but it has been suggested that competition for the type I receptor might modulate the amplitude of the biological response (Schofield *et al.*, 1994). IGF II exerts a wide range of biological activities in cells in culture. It can promote cell proliferation by acting on the chromosome cell cycle (ie DNA-replication and mitosis) as well as on the cell growth cycle (cellular enlargement) (Dafgård *et al.*, 1986; Zetterberg *et al.*, 1982, 1984). IGF II can induce differentiation *in vitro*, an effect which has been characterised in detail in myoblasts (Florini *et al.*, 1991). IGF II can counteract apoptosis in some cell systems and thereby enhance survival (Granerus *et al.*, 1995; Granerus and Engström, 1996), whereas in other cell lines there appears to be an apoptosis inducing effect by IGF II (Granerus *et al.*, 1998). IGF II release also induces a functional modulation of certain cell types. It stimulates hormone synthesis and secretion in ovarian granulosa and theca cells (Giudice, 1992). It also binds to the type I receptor and thereby potentiates the release of histamine from basophils in response to immunoglobulin E (Hirai *et al.*, 1993). Finally, it has been shown that IGF II can stimulate motility in cultured rhabdomyosarcoma cells (Minitti *et al.*, 1992).

Overexpression of IGFs in transgenic mice has resulted in altered growth properties. Increased expression of an IGF I transgene leads to increased bodyweight and a limited overgrowth. Different tissues responded differently and growth disturbances and tumour formation were sometimes observed (Bol *et al.*, 1997; Coleman *et al.*, 1995; Matthews *et al.*, 1988; Reiss *et al.*, 1996). In several experimental situations, prolonged IGF II expression from transgenes using tissue-restricted regulatory elements has led to organ overgrowth and/or tumour formation (Bates *et al.*, 1995; Rogler *et al.*, 1994; Rossetti *et al.*, 1996; van Buul-Offers *et al.*, 1995; Ward *et al.*, 1994). More generalised IGF II overexpression has been achieved by introducing additional copies of the IGF II gene into embryonic stem cells, which were then used to generate chimaeric mice. An alternative approach to study how increased levels of IGF II can affect overall growth properties was to assay double mutant mice carrying a deletion around the H19 region as well as a targeted IGF type 2 receptor allele. Such mice have extremely high levels of IGF II and display most of the clinical features of the Wiedemann-Beckwith syndrome, as well as skeletal defects and a cleft palate: features of Simpson-Golabi-Behmel syndrome (Eggenschwiler *et al.*, 1997). In both these models of more general overgrowth, the affected animals die perinatally, thus making it impossible to assess their susceptibility to neoplasms. The development of transgenic technology has also rapidly made it possible to examine the effects of growth factor deficiency *in vivo*. When a disrupted IGF II gene was indroduced into the mouse germ line, the prenatal growth rate decreased and the body weight at term only reached 60% of the normal birth weight. However, the growth rate post partum appeared to be normal (deChiara *et al.*, 1990). Likewise, knockout-mice carrying null mutations for the IGF I gene lead to a significantly decreased birthweight, but with otherwise normal body proportions . Unlike the IGF II deficient mice, these transgenic animals had a decreased postnatal growth rate and a high degree of neonatal lethality (Baker *et al.*, 1993; Liu *et al.*, 1993).

The glucocorticoids and their receptors

Glucocorticoid hormones are biologically active steroid compounds that bear a close structural resemblance to one

another. Their biosynthesis is regulated by the hypothalamo-pituitary-adrenal axis which includes a number of intrinsic feedback mechanisms. In brief, a small peptide-corticotropin releasing factor (CRF) is secreted vy the hypothalamus and induces the synthesis of adrenocorticotropic hormone (ACTH) from the pituitary gland. ACTH subsequently stimulate the production and release of cortisol and other steroids from the adrenal cortex. Increased levels of cortisol also act as a feedback mechanism, whereby CRF and ACTH are inhibited.

Glucocorticoids induce glucose biosynthesis as well as exerting a variety of anabolic and catabolic effects. High levels of glucocorticoids suppress inflammation and the host immune response. This capacity has made them useful in pharmacological treatment of autoimmune diseases and suppression of transplant rejection. The physiological relevance of these profound effects on the modulation of the immune system are debatable since only pharmacological doses of glucocrticoids tend to yield them.

Glucocorticoids are involved in the organisms response to stress. The role of glucocorticoids in this coontext is to ensure that the brain and other critical tissues are adequately supplied with glucose. Glucocorticoids hence do not counteract the stress itself but rather abrogate the body's harmful response to stress.

The glucocorticoids are by nature hydrophobic and therefore transported in the bloodstream complexed with transcortin or corticostreoid binding proteins (CBG). Once released from its binding protein, the glucoocorticoid enters the cell by diffusion or active transport. In the cytoplasm, glucocorticoids bind reversibly to a specific glucocorticoid receptor (see Wright *et al.*, 1993 for a review). This receptor is maitained in an inactive state in a multiprotein complex, consisting of one receptor molecule and several heat shock proteins including hsp90, hsp70 and hsp56. Upon binding of the glucocorticoid, a dimer of hsp90 proteins is released from the complex whereby the glucocortricoid receptor acquires an increased affinity for DNA. The activated receptor dimerises before binding to specific sequences in the DNA. The glucocorticoid can alter the expression of target genes through at least three different mechanisms; (i) recruitment of the general transcriptional machinery (ii) modulation of transcription factor action, independent of DNA binding, through direct protein-protein interactions, and (iii) modulation of chromatin structure to allow the assembly of other gene regulatory proteins and/or general transcription machinery on the DNA. (McEwan *et al.*, 1997)

The specificity of glucocorticoid action is determined by well conserved DNA sequences which are bound by zinc finger domains within the activated receptor. The target DNA sequences are referred to as glucocorticoid response elements (GREs). A specific regognition site for the glucocorticoid receptor was first identified in the Mouse Mammary Tumour Virus long terminal repeats (MMTV-LTR). Subsequently, elements which mediate the action of glucocorticoids were found in the vicinity of a number of genes. By comparing the sequences a 15 base pair consensus motif was identified. It contains two partially palindromic hexamers (Zilliacus *et al.*, 1994) with a 3 base pair spacer in between. The conserved palindromic sequences as well as the spacer, which could consist of any three bases are critical for receptor mediated action *in vivo*. Activated glucocorticoid receptors, like other steroid receptors, bind to GREs and affect transcription. Therefore the hormonal response must be determined by the presence or absence of particular receptors and/or receptor specific requirements for additional factors to achieve transcriptional modulation within a given cell.

Considerably less is known about how DNA binding represses transcription than about how it activates it (see Dahlman-Wright, 1991 for review). Studies of a variety of genes that are downregulated by glucocorticoids suggest that the activated glucocorticoid receptor interferes with the binding or activity of other transcription factors.

A consensus sequence for receptor binding sites which mediate negative regulation—ATYACnnTnTGATCn—was proposed by Beato and co-workers (Beato, 1989). The biological significance of this element remains to be clarified.

One area that has attracted much attention has been the potential interplay between the signal transduction pathways mediating responses by the glucocorticoid receptor and the AP-1 family of transcription factors. It was shown that collagenase promoter activity is inhibited by glucocorticoids via the AP-1 site with a stringent requirement of glucocorticoid receptor. C-jun, which is one component of the AP-1 complex, inhibits the transactivation of the glucocorticoid receptor (Jonat *et al.*, 1990; Schule *et al.*, 1990; Yang *et al.*, 1990). These authors also demonstrated a direct interaction between c-jun and the glucocorticoid receptor where they were found to inhibit each others binding. C-fos, another component of the AP-1 complex, was found to transrepress the glucocorticoid receptor in a similar fashion (Lucibello *et al.*, 1990). Subsequently a distinct modulating domain was identified in glucocorticoid receptor monomers that repress theactivity of the transcription factor AP-1 (Heck *et al.*, 1994).

The effects of glucocorticoids on IGF transcription

The combined effects of insulin like growth factors and glucocorticoid hormones on cell proliferation has been known for some time (Concover *et al.*, 1983). However, a direct effect of glucocorticoid administration on the transcriptional activity of the IGF II gene was first documented by Beck *et al.* (1987, 1988) and Levinovitz and Norstedt (1989). In both studies neonatal rats were injected with the cortisone analogue dexamethasone and the resultant effects on IGF II transcription in the liver were monitored. The reduction in IGF II transcription levels was dramatic which lead the authors to suggest a general down-regulatory action of glucocorticoid hormones on IGF II transcription. Similar results have since been reported in a variety of biological systems. Administration of the naturally occurring active steroid hormone corticosterone resulted in a rapid and significant decrease in hepatic mRNA levels in neonatal rats (Kitriki *et al.*, 1992). Further support for this notion was given in a study in which a patient with non-islet cell tumour hypoglycaemia was treated

with prednisolone following which IGF II production was suppressed (Baxter, 1996). In other animals, profound effects of glucocorticoid adminstration on the IGF II gene has been demonstrated. In sheep, infusion of cortisol as well as ACTH led to a decrease in IGF II expression in fetal adrenal glands (Lu *et al.*, 1994). Moreover, it has recently been shown that infusion of cortisol to fetal sheep during late gestation also results in a significant down-regulation of hepatic IGF II mRNA abundance (Forhead *et al.*, 1998). This effect was shown to be mediated by a specific suppression of the ovine P4 promoter (Li *et al.*, 1998). Pregnant minks that were treated with polychlorinated biphenyls increased their endogenous production of glucocorticoids which resulted in a decrease of IGF II transcription in the maternal liver (Bäcklin *et al.*, 1998). It has been speculated whether the temporary release of cortisol which normally occurs soon after birth in fact is responsible for their normal post-natal decline in IGF II transcription (Dalle *et al.*, 1985). The studies involving glucocorticoid administration during pregnancy suggested that this control point could be brought forward to an earlier developmental stage. Downregulation of gene transcription by glucocorticoids, has also been observed in the IGF I gene. Dexamethasone was shown to down regulate IGF I mRNA levels in rat neuronal and glial cells *in vitro* (Adamo *et al.*, 1988). Cortisol decreased the transcriptional activity of the IGF I gene in human osteoblast cells in vitro (Swolin *et al.*, 1996).

The generality of the concept that glucocorticoids suppress IGF gene expression has been challenged several times over the last decade. For instance it was shown that dexamethasone treatment of pregnant rats resulted in a limited but significant increase in IGF II mRNA levels in fetal livers (Price *et al.*, 1992). There seemed to be a certain amount of organ specificity, since lung tissues taken from the same animals appeared to contain similar levels of IGF II transcript whether or not their mothers had received glucocorticoid treatment (Price *et al.*, 1992). In a series of adult male volunteers, dexamethasone did not affect serum levels of IGF II, but this result does not exclude local differences in transcriptional activity between different organs (Miell *et al.*, 1994). Interestingly, several cell types grown *in vitro* increase their production of IGF II mRNA in response to glucocorticoid addition to the culture medium including pheochromocytoma cells (Liu *et al.*, 1994) and mouse myogenic cells (Yoshiko *et al.*, 1998). When pregnant sows were injected with either dexamethasone or hydrocortisone their first trimester fetuses contained increased quantities of IGF II mRNA driven from the fetal promoters (Madej *et al.*, 1996; unpublished).

The up-regulation of IGF II expression in response to glucocorticoids is difficult to square with the absence of obvious GREs in some of the IGF II promoter regions. However, it has recently been shown that an isolated IGF II P3 promoter construct can be activated by glucocorticoids in the absence of a GRE. The augmentation of this stimulatory effect by other flanking enhancer elements pointed at the possible existence of indirect mechanisms for glucocorticoid induced gene activation. In principle, this is testable in cell culture systems where simultaneous addition of hormone and protein synthesis inhibitors would ameliorate any indirect effects on IGF expression. It should be noted, however, that there seems to exist a coordinated regulation of glucocrticoid biosynthesis and IGF gene expresion, at least in the embryo (Yuan *et al.*, 1996). Also, insulin-like growth factors appear to exert an effect on steroid production, thereby providing evidence for further complexity in steroid-IGF interplay (Mesiano *et al.*, 1997)

IGF binding proteins and glucocorticoids

IGF binding proteins are secreted proteins which comprise a major regulatory component of the IGF signalling pathway (Cohick and Clemmons, 1993). Six IGFBPs have so far been characterised; all bind IGFs with high affinity and are capable of modifying the biological actions of IGFs within tissues as well as transporting the IGFs between body compartments. They are separate gene products, but much of the amino acid sequence and multiple cystine bridges are conserved among the different IGFBPs (Drop *et al.*, 1992; Shimasaki and Ling, 1991). Each IGFBP has a distinct tissue-specific and developmentally regulated expression pattern (Cheung *et al.*, 1994; Lindenberg-Kortleve *et al.*, 1997; Shimasaki and Ling, 1991), as well as differing affinities for the IGFs. This has led to the belief that each IGFBP has a specialised role in each tissue, modulating the activity of IGF I and II in a negative (complexing with IGFs to limit availability of free ligand for interaction with its receptor) or a positive (facilitating interaction with the receptor) manner. For example, IGFBP-6 binds IGF II with high affinity and prevents IGF II-mediated effects (Gabbitas and Canalis, 1997), whereas IGFBP-3, which is held to be the major carrier protein for IGFs, can either inhibit or potentiate actions of IGFs depending on the target cell type (Clemmons, 1992). It should also be noted that at least some of the IGFBPs may also exert effects that are independent of IGFs.

We have already reviewed the direct effects that glucocorticoids have on IGFs. As the IGFBPs clearly have an important part to play in regulating IGF activity, we should also consider whether glucocorticoids can regulate IGFs indirectly by regulating IGFBPs. This is indeed the case, especially so in tissues where IGFs are developmentally important including bone, muscle, and liver. As IGFBPs can either inhibit or potentiate IGF actions, it should be no surprise that they can be regulated in different directions by the same compound in different cell types. The interaction between glucocorticoids (mostly dexamethasone) and IGFBPs is better documented for some members of the family than others.

A. Glucocorticoids and IGFBP-1

The observation by Price *et al.* (1992) that treatment of pregnant rats with dexamethasone led to an 8-fold increase in IGFBP-1 mRNA expression in foetal livers and a smaller increase in foetal lungs was corroborated by *in vitro* experiments using primary rat hepatocytes (Miura *et al.*, 1992; Robertson *et al.*, 1994), human (Suwanichkul *et al.*, 1994) and rat hepatoma cells (Goswami *et al.*, 1994; Orlowski *et al.*,

1989; Suh *et al*., 1996). In rat foetal osteoblasts also demonstrated a rise in IGFBP-1 mRNA and protein after dexamethasone treatment, suggesting that IGFBP-1 can modulate local IGF actions on bone formation in response to changes in glucocorticoid concentration. Similar regulation has been observed in foetal osteoblasts (Concover *et al*., 1995). Also, cortisol levels in cord blood and IGFBP-1 levels were found to be directly correlated in newborn infants (Concover *et al*., 1996). Further work has been done to elucidate the mechanism of the regulation. In cells, dexamethasone regulated IGFBP-1 at the level of transcription by interacting with a GRE at –91/–77 relative to the start site of transcription of the IGFBP-1 promoter (Suh *et al*., 1996), although another putative GRE was identified further upstream (99). This GRE is essential for dexamethasone-stimulated activity; its deletion reduced transcription to basal levels. However, this GRE is of low affinity and for maximal basal and glucocorticoid-stimulated activity, an insulin response element, HNF-1 site and an AP-2 site are also necessary (Goswami *et al*., 1994; Robertson *et al*., 1994; Suh *et al*., 1996, 1997; Suwanichkul *et al*., 1994). As IGFBP-1 is unique among IGFBPs in its rapid regulation to metabolic and hormonal changes (Suh *et al*., 1997), it is not surprising to find that its regulation is influenced by insulin and glucocorticoids.

B. Glucocorticoids and IGFBP-2

Much less is known about the regulation of IGFBP-2 by glucocorticoids. However, an important interaction between the two was shown by Mouhedienne *et al*. (1996). Glucocorticoids are thought to be involved in lung maturation; IGFs are known to be important in development and differentiation. Incubation of rat type 2 stem cells of the alveolar epithelium with dexamethasone showed a marked increase, again at the transcriptional level, of IGFBP-2 mRNA and protein. In a key *in vivo* study (Price *et al*., 1992), IGFBP-2 was also found to be increased in foetal lung. An *in vitro* study (Mouhedienne *et al*., 1996) showed that an increase in IGFBP-2 correlated with a decrease in DNA synthesis. On further investigation of the regulation, a putative GRE was found in the IGFBP-2 promoter. It is not known how IGFBP-2 affects IGF II in this system, although the up-regulation of IGFBP-2 was accompanied by an increase in IGF II and IGF2R, It is plausible that glucocorticoid directs the lung towards differentiation rather than growth through IGF II. IGFBP-2 expression and mRNA levels were also found to be upregulated in pancreatic cell lines after treatment with dexamethasone (Katz *et al*., 1997); this could also indicate a role in differentiation control for this IGF binding protein.

C. Glucocorticoids and IGFBP-3

The majority of total IGFs circulate as a complex with ubiquitously expressed IGFBP-3; formation of this complex alters IGF distribution and clearance, and modifies IGF bioactivity (Villafuerte *et al*., 1995). IGFBP-3 levels are also modulated by a dynamic balance between soluble and membrane-bound protein (McCusker *et al*., 1990). Bearing this in mind, it is not surprising that glucocorticoids have been reported to up- and downregulate this protein in different tissues. Hepatic IGFBP-3, which probably represents the majority of circulating IGFBP-3, is downregulated after exposure of primary rat hepatocytes (co-cultured parenchymal and non-parenchymal cells) (Villafuerte *et al*., 1995). This regulation was dose-dependent using physiological (10–10, 10–8M) and pharmacological (10–6M) levels of dexamethasone. No GRE was found in the IGFBP-3 promoter, thus an indirect regulation was proposed. Dexamethasone was also found to decrease IGFBP-3 in human osteoblast cultures (Chevalley *et al*., 1996). In bone, IGFBP-3 enhances IGF actions and this was thus proposed as a mechanism by which glucocorticoids could inhibit bone formation by inhibiting IGF anabolic activity.

In dermal papilla explants, however, dexamethasone was reported to increase IGFBP-3 levels eightfold (Hembree *et al*., 1996). As IGFs are involved in hair follicle elongation, and glucocorticoids have been reported to suppress hair growth, it was proposed that in this system IGFBP-3 is acting to inhibit the mitogenic effects of IGFs, presumably by sequestering free IGF. In contrast to the results of Villafuerte *et al*. (1995), an earlier *in vivo* study showed an upregulation of IGFBP-3 at the protein and mRNA levels in rats treated with dexamethasone (Luo and Murphy, 1990), pointing at a mechanism for dexamethasone-induced growth retardation. Both serum and hepatic levels were affected. This difference may purely be due to the difference between an *in vivo* and an *in vitro* system, and does indeed point to an indirect effect of glucocorticoids on IGFBP-3.

D. Glucocorticoids and IGFBP-4

A different and rather attractive mechanism has been proposed for glucocorticoid regulation of IGFBP-4, which has been postulated to be important in the nervous system (Cheung *et al*., 1994). When dexamethasone was applied to cultures of rat neuronal cells (Cheung *et al*., 1994), abundance of native IGFBP-4 protein dropped to 10% of control levels. Unusually, this was not accompanied by a change in mRNA levels but an increase in a breakdown product of IGFBP-4 was observed. Dexamethasone had induced a protease which specifically cleaves IGFBP-4, thus reducing native levels and increasing levels of a smaller form with lower affinity for IGFs. It is difficult to speculate on how this would affect IGF actions; IGFBP-4 has been shown in vitro to inhibit IGFs, presumably because its affinity for IGFs is higher than that of the IGF1 receptor (Orlowski *et al*., 1989). Thus, proteolysis of IGFBP-4 could permit IGF action by allowing access of IGF to its receptor (Cheung *et al*., 1994). Dexamethasone has also been shown to inhibit basal IGFBP-4 secretion in bovine and human fibroblasts (Concover *et al*., 1995), but here an effect on mRNA levels was also observed, indicating a different mechanism at work. In cultured pancreatic cells, however, dexamethasone stimulated secretion of IGFBP-4 (Katz *et al*., 1997). This would appear to povide further evidence for a complex cell-specific regulation of IGFBPs by glucocorticoids.

E. Glucocorticoids and IGFBP-5

Again, not much is known about this IGFBP regarding its physiological role. It is thought to enhance IGF actions, and its expression was found to be decreased after treatment of human osteoblasts with dexamethasone (Chevally *et al.*, 1996). This, together with the regulation already described of IGFBP-3 and IGFBP-4 in this system, suggests a role for IGFBPs in controlling IGF activity in bone formation.

F. Glucocorticoids and IGFBP-6

IGFBP-6 binds IGF II with high affinity (at least 20 times higher) than IGF I and prevents IGF II-mediated effects on myoblasts and osteoblasts (Bach *et al.*, 1993), indicating a role for this IGFBP in control of muscle and bone differentiation in fetal growth (Gabbitas and Canalis, 1997). Glucocorticoids induce the expression of IGFBP-6 in cultured foetal rat osteoblasts (Gabbitas and Canalis, 1996) : cortisol induced a time- and dose-dependent increase in IGFBP-6 mRNA and protein, at the transcriptional level, indicating a possible mechanism for the inhibitory effects of glucocorticoids on bone formation. In contrast, though, dexamethasone decreased IGFBP-6 protein and mRNA levels in rat PC12 phaeochromocytoma cells, also at the level of transcription (Bach *et al.*, 1993). This cell-specific regulation, coupled with the fact that glucocorticoids induce differentiation of PC12 cells to a chromaffin rather than a neural phenotype, suggests that IGFBP-6 and the IGF system may be involved in chromaffin differentiation of these cells.

Glucocorticoids and IGF receptors

Considering the importance of the IGF receptors in the IGF signalling system, surprisingly little is known about their regulation by glucocorticoids. The *in vivo* study conducted by Price *et al.* (1992) in rats showed that exposure of fetuses to dexamethasone resulted in increased IGF1R mRNA levels in liver and lung (the two tissues examined), suggesting that decreased receptor availability does not contribute to dexamethasone-induced growth retardation. Two interesting effects of glucocorticoids on IGF receptors have been documented. Firstly, in rat fetal osteoblasts, cortisol did not change IGF1R mRNA levels but did time-and dose-dependently transcriptionally decrease IGF2R mRNA and protein levels (Rydziel and Canalis, 1995). IGF2R is thought mainly to be a sink for IGFs, lowering free IGF levels, so this result, which would have the effect of raising free IGF levels, is intriguing. Secondly, two contrasting but similar effects of dexamethasone have been observed. Dexamethasone blocked the IGF I-induced increase in IGF binding in rat chondrocytes, without having any effects on basal IGF1R levels (Jux *et al.*, 1998). In porcine ovary granulosa cells, however, dexamethasone prevented the normal decrease in IGF1R levels induced by IGF I (Urban *et al.*, 1994), effectively raising IGF1R levels but with no change in mRNA or protein levels. These would appear to be indirect effects, dexamethasone blocking the effects of IGF I and thus affecting the receptor, and it would be interesting to ascertain the mechanism behind this.

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